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# ABSORPTION AND EMISSION SPECTRA OF PSORALEN AND 8-METHOXYPSORALEN IN POWDERS AND IN SOLUTIONS*

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The effects of certain of the furocoumarins in sensitizing organisms to near ultraviolet and visible radiation is well known (1, 2, 3). This paper will deal with the absorptive and emissive properties of two such compounds, psoralen (PS) and 8-methoxypsoralen (8-MOP).

The UV absorption spectra for these compounds have been reported by Fowlks (4): absorption maxima at about 220, 245, and 295 mμ (with a shoulder at 330) for PS and at about 220, 250, and 310 mμ for 8-MOP. Pathak *et al* and Pathak and Fellman (5, 6) reported that maxima in the excitation spectra for fluorescence at room temperature from both PS and 8-MOP occurred at 265–280 mμ and also at 340–380 mμ, whereas the excitation maxima for phosphorescence at 77° K occurred at 360 mμ (Some of these measurements did not include wavelengths below 310 mμ.). In spite of the fact that these values deviate markedly from the absorption maxima, Pathak and Fellman predicted that PS and 8-MOP should induce maximal photopathological effects when exposed to 360 mμ light (6). Magnus indeed later reported a maximum at about 360 mμ in the action spectrum for erythemal activity of 8-MOP on human skin (3). This latter author then concluded that if the findings of Pathak and Fellman "are confirmed they may make us use the Grotthus-Draper principle, the so-called first law of photochemistry, with more caution. Apparently, as far as human skin is concerned, the furocoumarin molecule is more biologically active when in a state of fluorescence rather than when in a state of absorbing light maximally."

If the correspondence cited in references 5 and 6 were valid, then some unusual physical principle might indeed be operative in the promotion of skin damage by the furocoumarins. However, many of the quantitative results presented by Pathak and co-workers (5, 6) were of only an approximate nature with few statements about limits of accuracy. In addition, certain of their qualitative results seemed to be both sufficiently important and tenuous to necessitate further investigation. Thus, it seems worthwhile to report our studies of the emissive properties of PS and 8-MOP, since we find that when excitation spectra are obtained in a *"front-face" cell* and are *corrected for differences in output of the exciting lamp* at different wavelengths, reasonable agreement is obtained between the positions of maxima in the spectra for absorption and for excitation. In particular, no maxima are apparent at 360 mμ. Although the quantum efficiency is not uniform for excitation throughout the absorption bands, our data do not indicate that these two important molecules are abnormal in their photochemical behavior. To determine whether unusual physical and/or chemical mechanisms must be invoked to explain their erythemal effects will require accurate measurements of biological action spectra with suitable adjustment for actual absorption within the biological material.

## MATERIALS AND METHOD

The solvent used in solution studies was a 50% aqueous-glycerol mixture; the water was doubly distilled in a pyrex container and the glycerol was of "spectro" grade obtained from Matheson, Coleman, and Bell Chemical Company. The preparation of the PS and 8-MOP and their solutions was similar to that described in references 4 and 5.

Emission spectra from both powders and $H_2O$-glycerol solutions of PS and 8-MOP were recorded in the wavelength range 200–800 mμ at room temperature and at 77°K. Emission and excitation measurements were made on an Aminco spectrophosphorimeter equipped with a special cuvette in which fluorescence was stimulated from the outer surface only. In the Aminco instrument two monochromators are situated at right angles to each other: one is used to select the wavelength for excitation and the other to select the emission wavelength. The exciting light is obtained from a Xenon gas lamp and the output is measured with a 1P28 photomultiplier tube. Corrections are made

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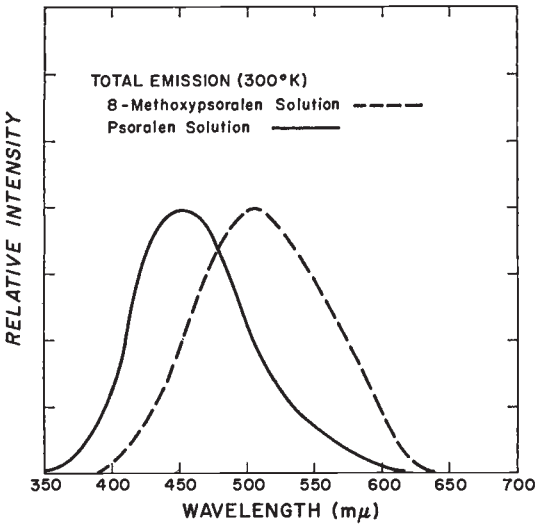


FIG. 1. The total emission spectra obtained at 300°K: psoralen in solution excited at 335 mμ and 8-methoxypsoralen in solution excited at 310 mμ. Both solutions contained $10^{-4}$ M solute in a 1:1 glycerol-water mixture.

in the excitation spectra for variations in the output of the Xenon lamp at different wavelengths and in the emission spectra for variations in the sensitivity of the photomultiplier at different wavelengths. This instrument has an overall accuracy of about 25 Å for both excitation and emission spectra. More complete details of this instrument have been published elsewhere (7).

Inasmuch as a rotating shutter must be inserted to measure phosphorescence, the absolute intensity of the phosphorescence alone usually cannot be compared directly with the intensities of the total emission curves. In some cases, however, such as in Figure 2, the correspondence between the shapes of the phosphorescence curve and the same wavelength region in the total emission curves is obvious.

The room temperature fluorescence quantum yields were calculated as follows:

Let

$Y_S$ = quantum yield of a given sample

$Y_Q$ = quantum yield of a quinine sulfate standard

$\frac{q_S^E}{q_S^A} = \frac{\text{number of quanta emitted by the sample}}{\text{number of quanta absorbed by the sample}}$

Then;

$$Y_S = \frac{q_S^E}{q_S^A} \quad \text{and} \quad Y_Q = \frac{q_Q^E}{q_Q^A}$$

When the optical density of the quinine sulfate standard is equal to the optical density of the sample solution then $q_S^A = q_Q^A$ for that wavelength and

$$Y_S = Y_Q \frac{q_S^E}{q_Q^E}$$

In the present experiments sample concentrations ($10^{-4}$ M) were chosen such that when $q_S^A = q_Q^A$, the concentration of quinine sulfate was small enough to neglect concentration quenching and $Y_Q = 0.55$ (8): four different concentrations of quinine sulfate were used to match the absorbances of the two compounds at the two wavelengths—250 and 340 mμ—investigated.

## RESULTS AND DISCUSSION

Figure 1 shows the room temperature emission from PS and 8-MOP in solution. Qualitatively identical results were obtained when the wavelength of the exciting light was that of the peak in either the uncorrected or the corrected excitation spectrum. This can be compared with the total emission spectra of the same solutions of 77° K shown in Figure 2. As listed in Table I the quantum yields of fluorescence for absorption at 250 mμ for PS and 8-MOP are 0.02 and 0.002, repectively, and for absorption at 340 mμ are 0.02 and 0.004, respectively. Note that at room temperature and at 77° K the fluorescence of PS appears at a higher energy than the fluorescence of 8-MOP. By contrast the phosphorescence spectra from these two solutions are almost identical in terms of both the shape of the spectra and the wavelengths at which emission occurs. Further, in both PS and 8-MOP solutions at low temperatures, the number of phosphorescence quanta greatly exceeds the number of fluorescence quanta. Contrary to earlier implications (5), it is not unusual to find phosphorescence in biochemicals at 77° K (see, for instance, ref. 7). At room temperature, however, phosphorescence is seldom seen; eosin is an exception (9). The lack of phosphorescence may not reflect that fewer triplet states are formed as reported by Pathak *et al* (5), but that at room temperature the triplet states are effectively quenched by collisions with other molecules. It is, in fact, entirely possible that more triplet states are formed *initially* at room temperature than at 77° K since at higher temperatures the molecules will possess greater vibrational energy which may *enhance* intersystem crossing from the singlet state to the triplet state configuration (10).

Figure 3 shows the total emission and phosphorescence spectra from PS and 8-MOP powders at 77° K. As in the case of solutions, the maximal fluorescence of PS appears at a

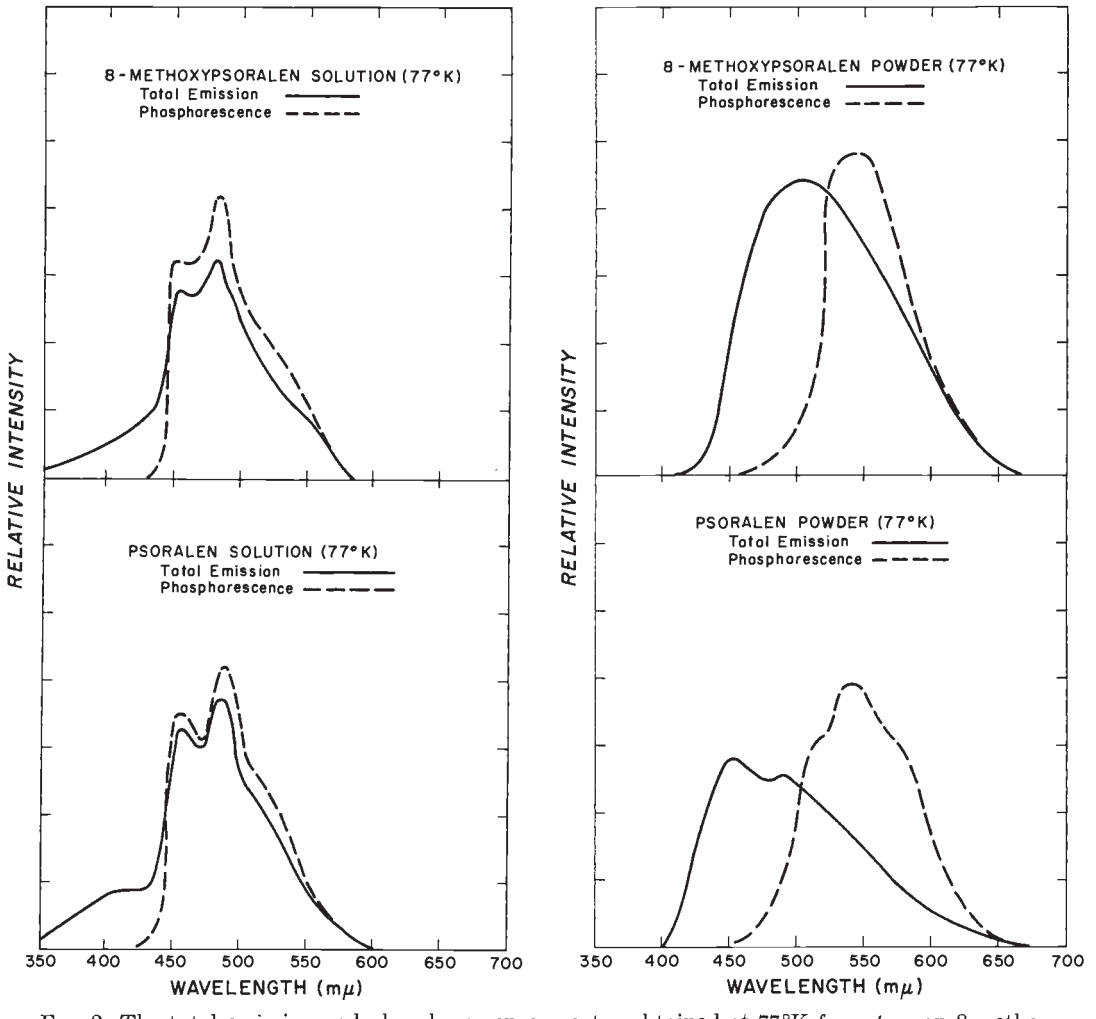


FIG. 2. The total emission and phosphorescence spectra obtained at 77°K from *top:* an 8-methoxypsoralen solution excited at 310 mμ and *bottom:* a psoralen solution excited at 335 mμ. Both solutions contained $10^{-4}$ M solute in a 1:1 glycerol-water mixture.

FIG. 3. The total emission and phosphorescence spectra obtained at 77°K from *top:* 8-methoxypsoralen powder excited at 400 mμ and *bottom:* psoralen powder excited at 370 mμ.

higher energy than that from 8-MOP while the phosphorescence maxima are again at the same wavelength. However, the fine structure—having a vibronic spacing of about 1300 $cm^{-1}$—seen in emission spectra from solutions is not discernable in the phosphorescence from the powders although there are distinct shoulders on the phosphorescence spectrum of PS powder which are separated from the maximum by approximately 1100–1200 $cm^{-1}$.

Figure 4 shows phosphorescence decay curves for PS and 8-MOP in powder form and in water-glycerol solutions. In three out of the four cases the straight lines obtained on the semilog plots indicate first order decay kinetics, *i.e.*, a single emitting species. In the case of PS powder the decay curve can be resolved into two first-order curves with mean decay times of 0.4 and 1.1 sec. While it might be inferred that lifetimes of about 1 sec. would indicate that molecules in triplet state configurations could have many opportunities for entering into reactions, it is necessary to bear in mind that the lifetime measurements were made at

TABLE I

| | Psoralen | 8-Methoxypsoralen |
|---|---|---|
| Quantum yield for fluorescence: | | |
| Solution, 300°K, absorption at 250 mμ | 0.02 | 0.002 |
| Solution, 300°K, absorption at 340 mμ | 0.02 | 0.004 |
| Emission maxima: | | |
| Solution, 300°K | 450 mμ | 505 mμ |
| Solution, fluorescence, 77°K | *ca.* 400 mμ | *ca.* 425 mμ |
| Solution, phosphorescence, 77°K. | 455, 485 mμ | 455, 485 mμ |
| Powder, fluorescence, 77°K | 450 mμ | 475 mμ |
| Powder, phosphorescence, 77°K | 540 mμ | 540 mμ |
| Absorption maxima: | | |
| Solution, 300°K | 245, 295 mμ | 220, 250, 305 mμ |
| Excitation maxima: | | |
| Solution, 300°K | 245, 305 mμ | 250, 305 mμ |
| Phosphorescence decay times ($t_{37}$): | | |
| Solution, 77°K | 1.1 sec. | 0.8 sec. |
| Powder | 0.4, 1.1 sec. | 0.5 sec. |

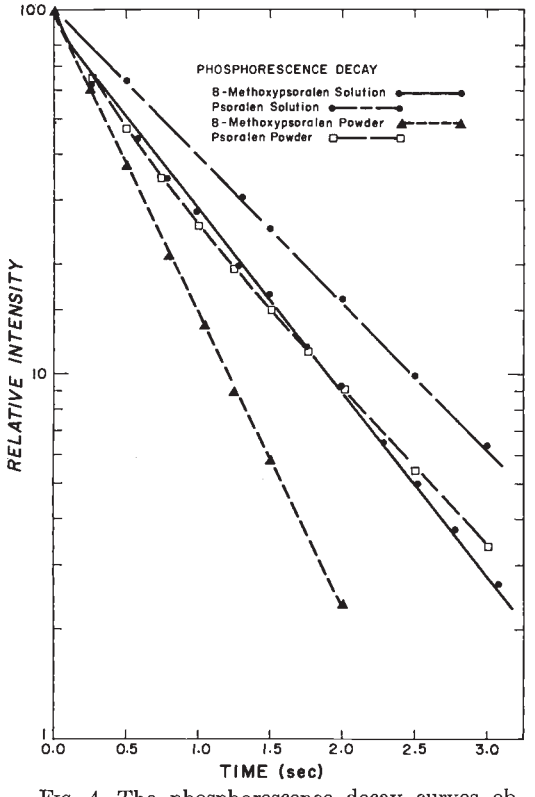


FIG. 4. The phosphorescence decay curves obtained at 77°K from $10^{-4}$ M 8-methoxypsoralen solution, $10^{-4}$ M psoralen solution, 8-methoxypsoralen powder, and psoralen powder.

77° K. At room temperature, we could detect no phosphorescence with our instrumentation indicating that the intrinsic triplet lifetime at room temperature can not be greater than $10^{-3}$ sec., and may be much less. A tabular summary of the results of the present work is given in Table I.

The results for PS and 8-MOP presented here differ from earlier work (5, 6). Possible explanations for these differences are as follows:

1. The earlier workers measured phosphorescence with a spectrofluorimeter. The fact that this instrument does not have a rotating shutter means that measurements of phosphorescence are likely to have a large error for a number of reasons: one incidental factor is the instability of the lamp output over long measuring times.

2. No mention was made in the earlier work of corrections for variations in the output of the exciting lamp or in photomultiplier response at different wavelengths. The latter variations, which apply to emission spectra, are not too great in the wavelength range 300–550 mμ, being between one and two. However, the variations in the output of the exciting lamp are quite large; the correction factor at 200 mμ is 32 compared to 1.0 at 350 mμ. Failure to apply these corrections can easily result in excitation spectra which show a misleadingly large amplitude between 300 and 400 mμ.

3. The type of sample container used in the fluorescence excitation work of the earlier papers can cause an excitation artifact in solutions with a large optical density; that is, the part of the sample from which fluoresence

is observed is at the center of the cuvette. As a consequence, wavelengths which are absorbed the most strongly are diminished in intensity before reaching the center of the solution in the cuvette. This gives the illusion of a relatively strong ability to excite fluorescence by wavelengths which are not strongly absorbed. In the present work, this difficulty has been partially alleviated by the use of a special cell which permits observation of emission initiated primarily at the *front surface* of the cuvette before the spectral composition of the exciting light has been distorted by excessive absorption in passing through the solution.

Earlier papers (5, 6) report that maximal phosphorescence and fluorescence excitation occurred at 360 mμ. Examination of the absorption spectra of these compounds in either Figure 5 or in the report of Fowlks (4) shows that the absorption of PS and 8-MOP near 360 mμ is less than 20% of the absorbance at 300 mμ and less than 10% of that at 245 mμ. Furthermore, there is no absorption peak near 360 mμ for either compound. The peaks in the excitation spectra for both fluorescence and phosphorescence from PS and 8-MOP which have been corrected for lamp output agree with the peaks in the absorption spectra shown in Figure 5. This figure also contains uncorrected excitation and corrected excitation curves for PS and 8-MOP made with a "front-face" cell and an uncorrected excitation spectrum made with the standard type of four-sided quartz cell which is 1 cm on each side.

4. The earlier workers reported data for fluorescence at room temperature and phosphorescence at low temperatures. The work presented here shows that the room temperature fluorescence and the low temperature (77° K) fluorescence do not exhibit maxima at the same wavelength and are, therefore, not interchangeable.

An ultimate aim of spectroscopic work on the furocoumarins is to ascertain the mechanisms by which this group of compounds induce photopathological effects. Magnus (3) has referred to work which shows that the maximum erythemal effect induced by 8-MOP occurs at about 360 mμ. Thus, perhaps the photopathological action spectrum and the fluorescence action spectrum for 8-MOP are not the same. However, the implications of such a conclusion are so important that further

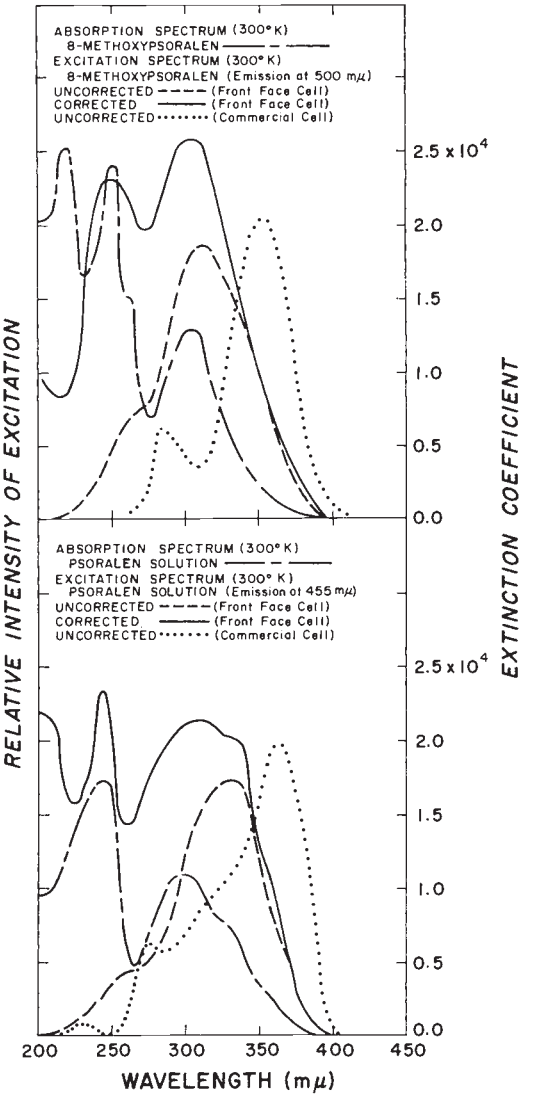


FIG. 5. Absorption spectra, uncorrected excitation spectra obtained with a "front-face" cell, corrected excitation spectra obtained with a "front-face" cell, uncorrected excitation spectra measured in a commercial cell. All of these spectra were obtained at 300°K from $10^{-4}$ M *top:* 8-methoxypsoralen and *bottom:* psoralen in 1:1 glycerol-water mixture.

investigation is warranted. Even more accurate action spectra for erythemal activity must be obtained; in particular, technics for the very accurate quantitative measurement of the degree of injury are needed. It might be possible to measure the extent of injury in the following manner: Color transparencies would be taken of the skin before and after light exposure.

Absorption spectra of the latter transparency, using the former as a blank, would then provide a means of quantitating injury.

If such measurements confirm the conclusions presented by Magnus (3) then it may be that one of three possible phenomena is taking place in photosensitization of skin by PS and 8-MOP:

a) The photosensitizing agent undergoes a drastic chemical change upon being incorporated into tissue.

b) Certain chemicals in the skin filter out light in the wavelength region 290–340 mμ before it reaches the site at which photopathological effects take place.

c) The photosensitizing agent reacts with other compounds upon being incorporated into tissue to form complexes having absorption maxima at 360 mμ.

A model similar to c) has been tested by Spikes and Ghiron (11) for the photosensitization of trypsin by riboflavin and flavin mononucleotide. They did not find differences in the fluorescence and absorption spectra (over a wide pH range) such as can be expected to be associated with the binding of flavins to proteins.

The results presented in this report suggest approaches to further experimentation in the field of photosensitizing action of the furocoumarins. Experimentation with light-produced intermediates of these compounds should be undertaken in a manner analogous to earlier studies of the photodynamic action of chlorothiazides (12). Investigation of the possibility of energy transfer between the furocoumarins (or compounds related to the furocoumarins) and critical cellular components might also suggest a mechanism to explain the erythemal activity of psoralen and 8-methxypsoralen.

SUMMARY

Absorption, total emission, and phosphorescence spectra of psoralen and 8-methoxypsoralen at 300° K and 77° K are presented; the various spectra were obtained from glycerol-water solutions of these compounds. Total emission and phosphorescence spectra from both compounds in powder form are also given. Psoralen and 8-methoxypsoralen in solution and in powder form at 77° K have phosphorescence lifetimes varying from 0.4 to 1.1 seconds. Room temperature fluorescence quantum yields for psoralen and 8-methoxypsoralen are 0.02 and 0.002, respectively, for absorption at 250 mμ and 0.02 and 0.004, respectively, for absorption at 340 mμ.

The differences in the results reported here and those of earlier workers could arise if they failed to correct for the errors inherent in the type of instrumentation used. In particular, when the corrections for output from the exciting lamp are made, the peaks of the room temperature fluorescence excitation spectra of psoralen and 8-methoxypsoralen obtained with a "front-face" cell agree with the peaks in their respective absorption spectra, contrary to earlier reports.

The maxima in the corrected excitation spectra given in the present report do not correspond to the maxima of the photopathological action spectra for these compounds. Thus, it is suggested that the photo-sensitizing agents, psoralen or 8-methoxypsoralen, may undergo a chemical change upon their incorporation into the skin or that the skin may filter out the wavelengths of light corresponding to the normal absorption maxima of those compounds.

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